

PATENT ABSTRACTS OF JAPAN

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C12N 5/10
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C12Q 1/68
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(C12N 5/10
C12R 1:91)
(C12P 21/02
C12R 1:19)

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KENKYUSHO:KK

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(54) FLUORESCENT PROTEIN

(57)Abstract:

PROBLEM TO BE SOLVED: To obtain a fluorescent protein capable of being expressed even by the culture of a host cell at a high temperature (37°C), emitting stronger fluorescent light than those of conventional fluorescent proteins (GFP), and useful as a labeling agent for the analyses of protein localization in live cells, a reporter for the analyses of promoters, etc., by introducing two mutation amino acids into a wild type GFP.

SOLUTION: This fluorescent protein is obtained by mutating the No. 147 serine and the No. 65 serine of the cDNA of a wild type GFP with proline and threonine, respectively, by a site-specific mutation method, etc., transforming *Escherichia coli* with a plasmid containing the obtained GFPcDNA and subsequently expressing the mutated GFP containing an amino acid sequence of the formula in the *Escherichia coli* at a high temperature (37°C). The fluorescent protein emits about three-fold fluorescent light that of S65T mutant.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240
 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270
 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300
 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330
 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360
 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390
 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420
 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450
 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480
 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510
 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540
 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570
 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600
 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630
 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660
 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690
 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720
 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750
 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780
 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810
 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840
 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870
 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900
 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930
 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960
 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990
 991 992 993 994 995 996 997 998 999 1000 1001 1002 1003 1004 1005 1006 1007 1008 1009 1010 1011 1012 1013 1

Tyr	Aen	C	Hf	Afr	Vat	T	Ple	Vat	Ala	Asp	Lys	Gly	Lys	Ser	M	
150			150						150						10	
His	Lys	Val	Asn	Thr	Lys	His	Arg	Ris	Asl	Ile	Glu	Arg	G	Y	Ser	Val
			160						170						170	
Glu	Leu	Ala	Asp	Ris	Tyr	Gln	Ala	Asp	Thr	Pro	Lie	Gly	Arg	Gly	Pr	
			18						185						150	
Val	Leu	Leu	Arg	Met	Asn	Ala	Tyr	Leu	Ser	Thr	Gln	Ser	Ala	Leu	As	
			195						210						205	
Lys	Asp	Pro	Asn	Tr	Lys	Val	Asp	His	Ser	Val	Leu	Pro	Glt	Pha	Val	
			210						215						220	
Tyr	Asp	Ala	Gly	Phe	Leu	His	Gly	Ser	Asp	G	Leu	Ser	Val			
			225						230						235	

is contained in a higher concentration than that of the S65T mutant, when expressed in the cell, and emits the fluorescent light under a high temperature (37°C).

LEGAL STATUS

[Date of request for examination]

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[Patent number]

[Date of registration]

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[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

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* NOTICES *

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1. This document has been translated by computer. So the translation may not reflect the original precisely.
2. **** shows the word which can not be translated.
3. In the drawings, any words are not translated.

CLAIMS

[Claim(s)]

[Claim 1] Fluorescence protein which includes an amino acid sequence of a publication in an array number 2.

[Claim 2] Fluorescence protein with which 1 or some amino acid include deletion and an amino acid sequence (however, the 65th place is a threonine and the 147th place is a proline) replaced or added in an array number 2 in an amino acid sequence of a publication.

[Claim 3] DNA which carries out the code of the fluorescence protein according to claim 1 or 2.

[Claim 4] A vector containing DNA according to claim 3.

[Claim 5] A vector according to claim 4 characterized by having arranged DNA according to claim 3 on a *-ed promotor's lower stream of a river.

[Claim 6] A host cell holding a vector according to claim 4.

[Claim 7] A manufacture method of fluorescence protein including a process which cultivates a host cell according to claim 6, and collects produced protein according to claim 1 or 2.

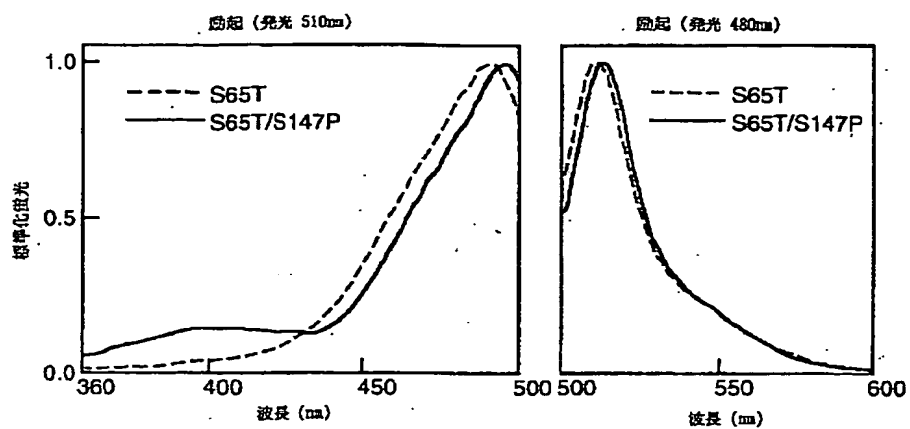
[Claim 8] A measuring method of the activity of a *-ed promotor who introduces a vector according to claim 5 into a host cell, and includes a process in which fluorescence emitted from this cell is detected.

[Claim 9] Fluorescence protein according to claim 1 or 2 characterized by uniting with a *-ed amino acid sequence.

[Claim 10] How to detect targetting activity in intracellular [of a *-ed amino acid sequence] which introduces fluorescence protein according to claim 9 into a cell, and is characterized by observing distribution in this intracellular one of this fluorescence protein.

[Claim 11] How to detect targetting activity in intracellular [of a *-ed amino acid sequence] which introduces into a host cell a vector in which DNA which carries out the code of the fluorescence protein according to claim 9 was inserted possible [a manifestation], and is characterized by observing distribution in this intracellular one of this fluorescence protein.

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Drawing selection drawing 1

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ITで消化した「S65T/S147P変異体」のcDNAを挿入し、マウス由来のL cellにカルシウム沈殿法で一過的トランスフェクトした。その細胞を37度で48時間培養した後に10%ホルマリンで固定し、蛍光顕微鏡によりノマルスキー(Nomarski)像およびFITCフィルターでの蛍光像(GFPの蛍光)を撮出した(図2A下段)。なお、対照として「S65T変異体」のcDNAを用いた(図2A上段)。この結果、「S65T変異体」と比較して、「S65T/S147P変異体」を発現する細胞は、より明るい蛍光像を示した(図2A右下)。

【0037】また、観察した細胞の内、蛍光を発する細胞の割合、及び細胞の蛍光の強さを測定した(図2B)。図の横軸は、最も蛍光の強かった細胞の蛍光強度を1とした場合における「S65T/S147P」は「S65T/S147P」は蛍光強度を示し、図の縦軸は、蛍光細胞の細胞数を示す。

【0038】この結果、「S65T/S147P変異体」のcDNAを挿入された細胞では、対照と比較して、より高い割合で細胞が蛍光を発していた。また、蛍光強度も対照と比較して顕著に高かった。

【0039】

【発明の効果】本発明により野生型GFPの65番目と147番目のアミノ酸がそれぞれトレオニン、プロリンに置換されたタンパク質が提供された。本発明のタンパク質は、37°Cの温度条件下においても蛍光型となり、また従来広く用いられてきた改良型GFPの約3倍の強い蛍光を発 *

*すると共に可溶性タンパク質としての発現量も2倍程度増加しているため、従来のタイプに比べ結果として37°Cで約5倍程度明るい蛍光を発することが明らかとなった。この改良型GFPは従来のものに比べ37°Cでの差が顕著であること、微生物のみならず動物細胞でも適用可能であることから、特に動物細胞や幅広い温度で生育可能な酵母などに有効と考えられる。本発明のGFPは、タンパク質の標識として用い、生細胞における分子の局在を観察する目的に適しているだけでなく、プロモーター解析におけるレポータータンパク質として、またタンパク質の高次構造変化のマーカーとしても有効と考えられ、今後広く細胞生物学、遺伝子工学分野においての利用が期待される。

【0040】

【配列表】

配列番号 : 1

配列の長さ : 717

配列の型 : 核酸

鎖の数 : 二本鎖

20 トポロジー : 直鎖状

配列の枠組 : cDNA to mRNA

配列の特徴

特徴を表す記号 : CDS

存在位置 : 1..714

特徴を決定した方法 : E

配列

ATG AGT AAA GGA GAA CAA CTT TTC ACT CGA GTT GTC CCA ATT CTT GTT	48
Met Ser Lys Gly Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val	
1 5 10 15	
GAA TTA GAT GGT GAT GTT AAT GGG CAC AAA TTT TCT GTC ACT GCA CAG	96
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu	
20 25 30	
GGT GAA GGT GAT GCA ACA TAC GGA AAA CTT ACC CTT AAA TTT ATT TCC	144
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys	
35 40 45	
ACT ACT GGA AAA CTA CCT GTT CCA TGG CCA ACA CTT GTC ACT ACT TTC	192
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe	
50 55 60	
TCT TAT GGT GTT CAA TCC TTT TCA AGA TAC CCA GAT CAT ATG AAA CCG	240
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg	
65 70 75 80	
CAT GAC TTT TTC AAG AGT CCC ATG CCC GAA GGT TAT GTA CAG GAA AGA	288
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg	
85 90 95	
ACT ATA TTT TTC AAA GAT GAC GGG AAC TAC AAG ACA CGT GCT GAA GTC	336
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val	
100 105 110	
AAG TTT GAA GGT GAT ACC CTT GTT AAT AGA ATC GAG TTA AAA GGT ATT	384
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile	

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11		12	
115	120	125	
GAT TTT AAA GAA GAT GCA AAC ATT CTT GCA CAC AAA TTG GAA TAC AAC			432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn			
130	135	140	
TAT AAC TCA CAC AAT GTA TAC ATC ATG GCA CAC AAA CAA AAG AAT GCA			480
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly			
145	150	155	160
ATC AAA GTT AAC TTC AAA ATT AGA CAC AAC ATT GAA GAT GCA AGC GTT			528
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val			
165	170	175	
CAA CTA GCA GAC CAT TAT CAA CAA AAT ACT CCA ATT GGC GAT GGC CCT			576
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro			
180	185	190	
GTC CTT TTA CCA GAC AAC CAT TAC CTG TCC ACA CAA TCT GCC CTT TCG			624
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser			
195	200	205	
AAA GAT CCC AAC GAA AAG ACA GAC CAC ATG GTC CTT CTT GAG TTT GTA			672
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val			
210	215	220	
ACA GCT GCT GCG ATT ACA CAT GCG ATG GAT CAA CTA TAC AAA			714
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys			
225	230	235	
TAA			717

配列番号 : 2

配列の長さ : 238

配列の型 : アミノ酸

* トポロジー : 直鎖状

配列の種類 : タンパク質

*

配列

Met Ser Lys Gly Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val		
1	5	10
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu		
20	25	30
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys		
35	40	45
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe		
50	55	60
Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg		
65	70	75
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg		
85	90	95
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val		
100	105	110
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile		
115	120	125
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn		
130	135	140
Tyr Asn Pro His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly		
145	150	155
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val		
165	170	175
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro		

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14

180 185 190
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195 200 205
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
210 215 220
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225 230 235

配列番号 : 3

* 配列の種類 : cDNA to mRNA

配列の長さ : 717

配列の特徴

配列の型 : 核酸

10 特徴を表す記号 : CDS

鎖の数 : 二本鎖

存在位置 : 1..714

トポロジー : 直鎖状

* 特徴を決定した方法 : E

配列

ATG AGT AAA GGA GAA GAA CTT TTC ACT CGA GTT GTC CCA ATT CTT GTT	48
Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val	
1 5 10 15	
GAA TTA GAT GGT GAT GTT AAT CCG CAC AAA TTT TCT GTC AGT GGA CAG	96
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu	
20 25 30	
GGT GAA GGT GAT CCA ACA TAC CGA AAA CTT ACC CTT AAA TTT ATT TGC	144
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys	
35 40 45	
ACT ACT CGA AAA CTA CCT GTT CCA TGG CCA ACA CTT GTC ACT ACT TTC	192
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe	
50 55 60	
ACT TAT GGT GTT CAA TGC TTT TCA AGA TAC CCA GAT CAT ATG AAA CCG	240
Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg	
65 70 75 80	
CAT GAC TTT TTC AAG AGT GGC ATG CCC GAA GGT TAT GTA CAG GAA AGA	288
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg	
85 90 95	
ACT ATA TTT TTC AAA GAT GAC CCG AAC TAC AAG ACA CGT GCT GAA GTC	336
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val	
100 105 110	
AAG TTT GAA GGT GAT ACC CTT GTT AAT AGA ATC GAG TTA AAA GGT ATT	384
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile	
115 120 125	
GAT TTT AAA GAA GAT CCA AAC ATT CTT CGA CAC AAA TTG GAA TAC AAC	432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn	
130 135 140	
TAT AAC CCA CAC AAT GTA TAC ATC ATG CCA CAC AAA CAA AAG AAT CCA	480
Tyr Asn Pro His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly	
145 150 155 160	
ATC AAA GTT AAC TTC AAA ATT AGA CAC AAC ATT GAA CAT GGA AGC GTT	528
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val	
165 170 175	
CAA CTA CCA GAC CAT TAT CAA CAA AAT ACT CCA ATT GGC GAT GGC CCT	576
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro	
180 185 190	
GTC CTT TTA CCA GAC AAC CAT TAC CTG TCC ACA CAA TCT GGC CTT TCG	624

(9)

符号平 1 0 - 2 3 4 3 8 2

15 16
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195 205 205
AAA GAT CCC AAC GAA AAG ACA GAC CAC ATG GTC CTT CTT GAG TTT GTA 672
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
210 215 220
ACA GCT GCT GCG ATT ACA CAT GCG ATG GAT GAA CTA TAC AAA 714
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225 230 235
TAA 717

配列番号 : 4

10*鎖の数 : 一本鎖

配列の長さ : 36

トポロジー : 直鎖状

配列の型 : 核酸

* 配列の種類 : 他の核酸 合成DNA

配列

GGCCCCGAT CCATGAGTAA AGGAGAAGAA CTTTTC 36

配列番号 : 5

*鎖の数 : 一本鎖

配列の長さ : 39

トポロジー : 直鎖状

配列の型 : 核酸

* 配列の種類 : 他の核酸 合成DNA

配列

GGCCACGGTA CCTTATTTGT ATAGTTCATC CATGCCATG 39

配列番号 : 6

20★鎖の数 : 一本鎖

配列の長さ : 31

トポロジー : 直鎖状

配列の型 : 核酸

★ 配列の種類 : 他の核酸 合成DNA

配列

TTCACCCGGG ATGAGTAAAG GAGAAGAACT T 31

配列番号 : 7

☆鎖の数 : 一本鎖

配列の長さ : 33

トポロジー : 直鎖状

配列の型 : 核酸

☆ 配列の種類 : 他の核酸 合成DNA

配列

GCACGAATTC TATTGTATA GTTCATCCAT GCC 33

【図面の簡単な説明】

30◆し、そのノマルスキー像及び蛍光像を示す顕微鏡写真で

【図1】「S65T/S147P変異体」及び「S65T変異体」の励起・蛍光スペクトルの測定結果を示す図である。

ある。図2Bは、被検細胞の中で蛍光を発する細胞の割合及びその細胞の蛍光の強さの測定結果を示す図である。

【図2】図2Aは、「S65T/S147P変異体」及び「S65T変異体」のcDNAが導入された細胞を蛍光顕微鏡により検出◆

【図1】

